Evolution of HIV-1 groups M and O: genetic comparative analysis of 23 HIV-1/MO inter-group recombinant forms

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Background

HIVs are characterized by a **high genetic diversity**, due to their simian origins and their replication mode, enhanced by recombination events. Despite the great genetic divergence between pandemic **HIV-1/M** and **HIV-1/O** (endemic in Cameroon), **dual infections** between the two groups can **generate HIV-1/MO inter-group recombinants**. Only three cases were described until 2004 [1-3]. Emergence of such mosaïc forms may have a strong impact on diagnosis and treatment due to the the presence of highly divergent group O fragments in their genome. Between 2006 and 2017, the implementation of a **sero-molecular algorithm in Cameroon** and the **exploration of atypical HIV-1 infection reactivities** at HIV diagnosis or during follow-up **in France** allowed the description of **new HIV-1/MO infections** [4-6].

Aim

Analyze and compare the **recombination patterns** and **genetic peculiarities** of all HIV-1/MO recombinants currently characterized.

Materials and methods

- **Collection of available data** on infected patients and on all recombinant forms characterized between 1999 and 2017: geographic origin of patients, partial/whole genome sequencing, possible co-infection with parental strains.
- **Comparison of frequencies of their characteristics**: phylogenetic analysis, breakpoints numbers in each mosaic genome and location.

Results

1. In addition to the first three cases described, **20 new recombinants or putative forms** were identified in Cameroon and France between 2006 and 2017. Thereby, **23 distinct, partially or completely sequenced, recombinant forms,** found in **22 patients,** were analyzed and compared, revealing a complexity of patterns [Fig.1].

2. This work also highlighted a **variable degree of complexity** in profiles, with on average **two to three** breakpoints per recombinant genome [Fig.1].

3. For the 23 MO recombinants, the **presence of HIV-1/M and/or HIV-1/O parental strains** was searched and results showed:
   - 12 recombinants with **no parental strains**
   - 10 recombinants **associated to one or two parental strains**
   - one case of **superinfection**, which led to the emergence of a recombinant form.

4. Phylogenetic analysis showed that the 23 recombinant forms corresponded to **19 distinct unique recombinant forms (URFs)** [Fig.2]:
   - two URFs in two pairs of patients without any epidemiological linked (BCF174/BCF212 and RBF222/RBF243 [6]),
   - one URF in two married patients (REC003/REC024 [4]),
   - one URF described by two different research teams, likely in the same patient (97CA.MP645MO [1]/ YBF298),
   - two URFs in the same patient (RBF221-1 and RBF221-2),
   - no known link for the 13 other URFs.

5. These 23 recombinants were all found in **patients of Cameroonian origin** and genetic diversity of the mosaïc fragments **matched the molecular epidemiology in Cameroon**. Indeed, a clear predominance of **HIV-1/M CRF02_AG** (53%) and of **HIV-1/O sub-group H** (87%) was observed [Fig.3].

6. Analysis of the genomic profiles and of the breakpoints frequency in the 23 described recombinant forms revealed [Fig.4]:
   - **hotspots in the "central" accessory genes** (*vif*, *vpr*, *vpu*), **reverse transcriptase** , **gp41** and **LTRs**
   - **no recombination event in protease**, **gp120** and ***nef***.

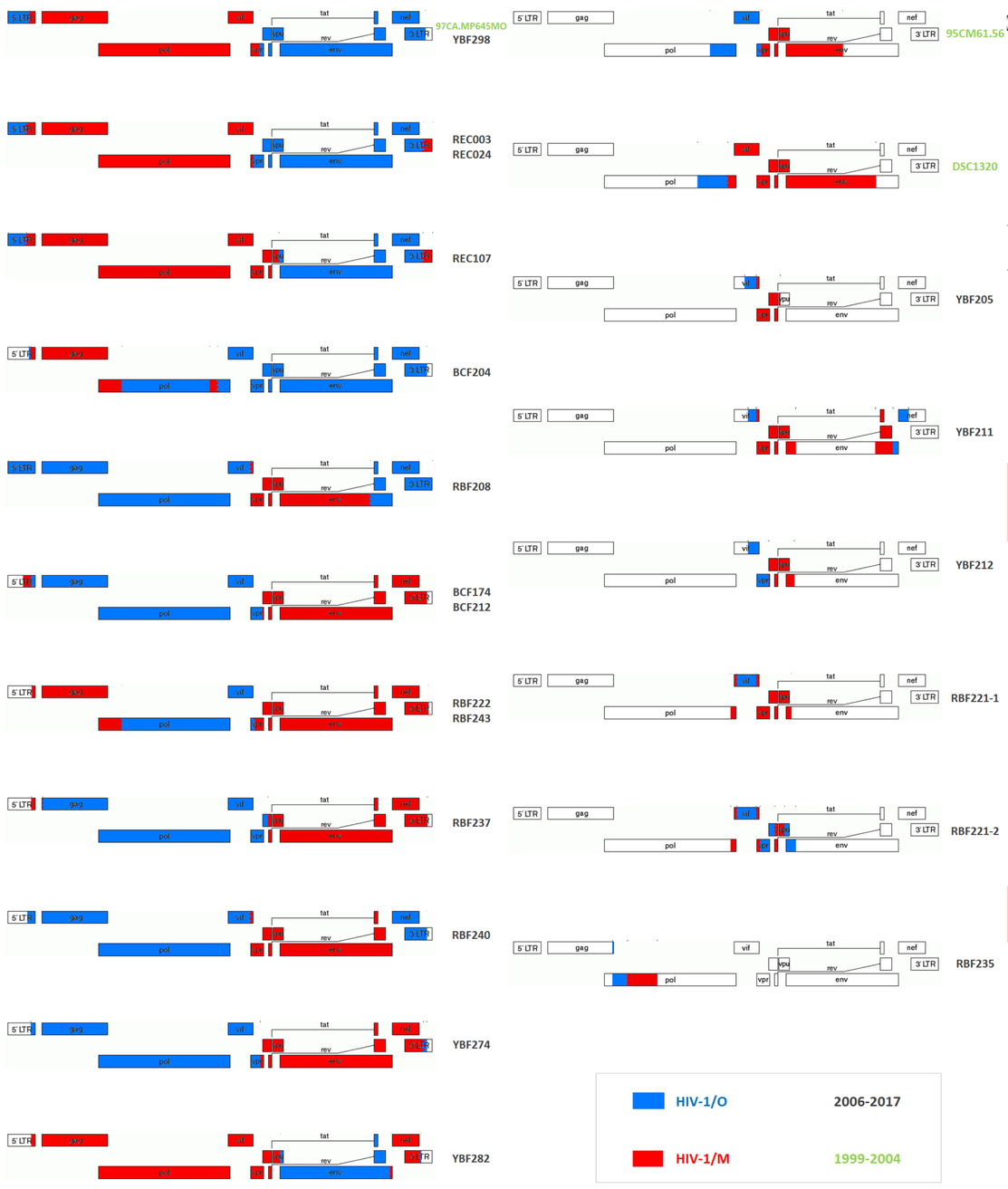

Fig. 1 Genomic profiles of the 23 described recombinants
Fragments belonging to HIV-1/O and /M are shown in blue and red respectively. Codes of recombinants described in 1999 and 2004 [1-4] are written in green and those of recombinants described between 2006 and 2017 in France and in Cameroon [4-6] are written in gray.

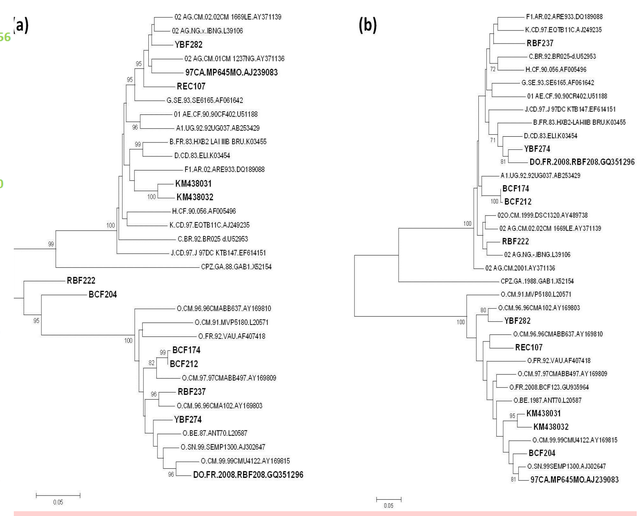

Fig. 2 Phylogenetic relationships of the HIV-1/MO fully sequenced Neighbour-joining trees of the PR-RT *[899bp]* (**a**) and gp41 *[443bp]* (**b**) regions were constructed with HIV-1/O and /M reference subset.

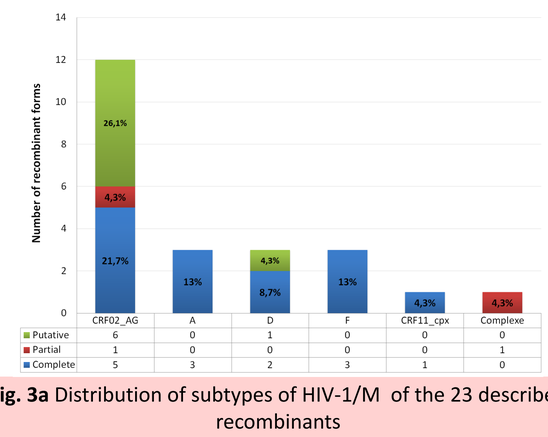

Fig. 3a Distribution of subtypes of HIV-1/M of the 23 described recombinants

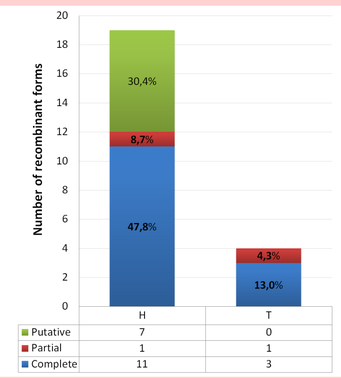

Fig. 3b Distribution of sub-groups of HIV-1/O of the 23 described recombinants [7]

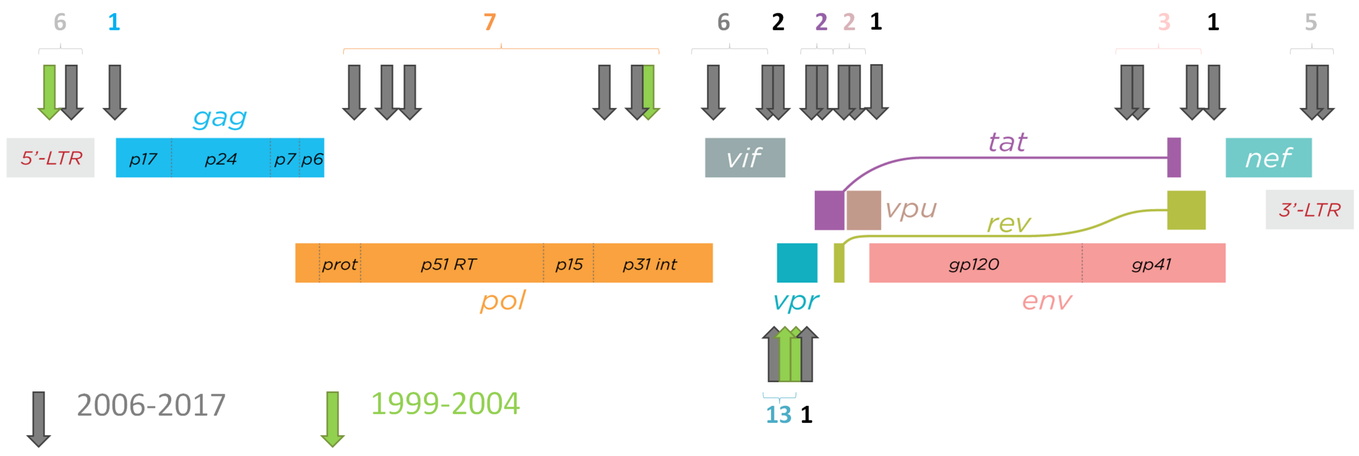

Fig. 4 Location of breakpoints identified in the genome of the 23 described recombinants
Arrows symbolize the location of the breakpoints in the genes, and numbers above indicate the number of breakpoints identified in the same region.
Green arrows represent the distribution of breakpoints along the genomes of the three recombinant forms described between 1999 and 2004 [1-3]. Gray arrows represent the distribution of breakpoints along the genomes of the 20 recombinant forms described between 2006 and 2017 in Cameroon and France [4-6].

Conclusions

- Despite the complexity of the recombination patterns, these recombinants could be detected with a **suitable sero-molecular strategy or following discordances** between detection and/or follow-up of results.
- The phenomenon of MO recombination is rare but **not anecdotal**, and **numerous situations** have been observed (single recombinants, or associated with one or two parental strains, or even a case of superinfection)
- **Inter-groups MO** recombination **hot/coldspot gradients** and the **location of breakpoints** were identified and remain to be compared with those in intra-group M recombinants.
- The analysis showed that recombinants were often single, with no parental strains, suggesting an **advantageous phenotype** of recombinant forms and a **possible better fitness** relative to parental strains, as observed by Peeters *et al.* [1].

In conclusion, this work allowed us to describe and analyze a unique series of HIV-1/MO recombinants, **highlighting a greater diversity and complexity than originally supposed**, and offering new research perspectives on the **conditions of emergence and virological properties of these recombinant forms**. Moreover, these multiple URFs-MO, single and transmitted in pairs of patients, could lead **to the diffusion and the emergence of a CRF_MO.**

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